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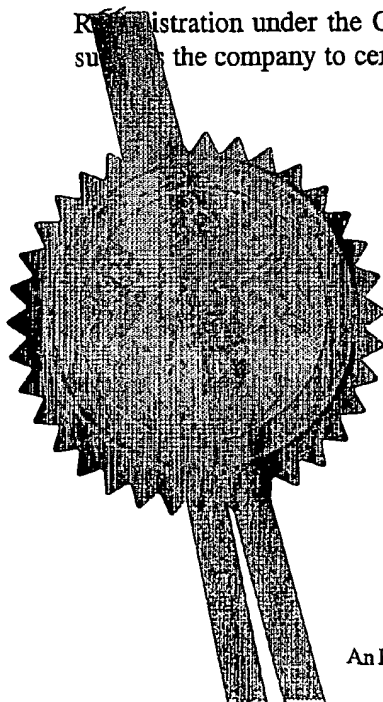
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*P. Mahoney*

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Dated 14 May 2003

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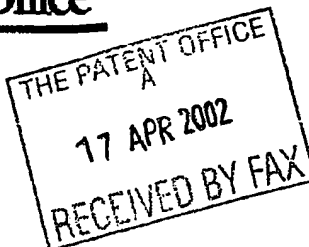
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17APR02 E711806-1 002866  
P01/7700 0.00-0208783.1

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)

Medical Research Council  
20 Park Crescent  
London W1B 1AL  
United Kingdom

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

596007001

4. Title of the invention

METHODS OF TREATMENT

5. Name of your agent (*if you have one*)

ERIC POTTER CLARKSON  
PARK VIEW HOUSE  
58 THE ROPEWALK  
NOTTINGHAM  
NG1 5DD

"Address for service" in the United Kingdom to which all correspondence should be sent (*including the postcode*)

Patents ADP number (*if you know it*)

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Date of filing  
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Number of earlier application

Date of filing  
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11.

I/We request the grant of a patent on the basis of this application.

Eric Potter Clarkson

Signature

ERIC POTTER CLARKSON

Date

17 April 2002

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## METHODS OF TREATMENT

The present invention relates to methods of treatment, and in particular methods of treating menorrhagia.

Menorrhagia is over-abundance of the menstrual discharge.

- 5 Menorrhagia affects many women, particularly in the Western world, and represent a significant health problem. At least one in 20 women in the UK aged between 34 and 49 years will consult their general practitioners because of menstrual problems. These women account for more than one in ten of all gynaecological referrals and cost the NHS in excess of £7 million per year for  
10 medical prescriptions alone. Perceived abnormal vaginal bleeding is said to account for 70% of the at least 70 000 hysterectomies done each year.

- At present, the treatments used for menorrhagia include tranexamic acid or mefenamic acid. In severe cases the treatment is hysterectomy (vaginal or abdominal) but this is a major operation with serious morbidity and some risk of  
15 death. A review of treatments for menorrhagia is Stirrat (1999) *The Lancet* 353, 2175-2176. The development of further and alternative therapies is desirable.

- The FP prostaglandin receptor has been studied in a variety of tissues including the bovine corpus luteum (Sharif *et al* 1998, *J. Pharmacol. Exp. Ther.* 286: 1094-1102); human uterus (Senior *et al* 1992, *Br. J. Pharmacol.* 108: 501-506);  
20 rabbit jugular vein (Chen *et al* 1995, *Br. J. Pharmacol.* 116: 3035-3041); various human ocular tissues (Davis & Sharif 1999, *J. Ocular Pharmacol. Ther.* 15: 323-336); and in mouse Swiss 3T3 fibroblasts (Griffin *et al* 1997, *J. Pharmacol. Exp. Ther.* 281: 845-854); and in rat vascular smooth muscle cells (A715) Griffin *et al* 1998, *J. Pharmacol. Exp. Ther.* 286: 411-418).

- 25 Potent, selective synthetic agonists at some prostaglandin receptors have been characterised in both *in vitro* and *in vivo* models (Coleman *et al* 1994, *Pharmacol. Rev.* 46: 205-229). For instance fluprostenol or its enantiomer (*eg*

AL-5848) (Sharif *et al.* 1999, *J. Pharm. Pharmacol.*, 51: 685-694) and cloprostenol (Coleman *et al.* 1994; Sharif *et al.* 1998) are potent and selective FP receptor agonists. Since most natural prostaglandins show rather limited selectivity for their preferred receptor among this receptor family, the few  
5 reported selective prostaglandin receptor agonists have been very valuable tools for discriminating discrete functional responses coupled to their respective receptors. However, conclusive identification of the particular receptors mediating prostaglandin-stimulated functional responses requires potent and selective antagonists (Kenakin 1996, *Pharmacol. Rev.* 48: 413-463).

10 The recent identification and commercial development of selective FP receptor agonists as potent and highly efficacious drugs for the treatment of elevated intraocular pressure (Bito 1997, *Surv. Ophthalmol.* 41 (Suppl. 22): S1-S14; Hellberg *et al.* 1998, *Invest. Ophthalmol. Vis. Sci.* 39 (Suppl.): 1961) has considerably advanced our knowledge of FP receptor-coupled pharmacological  
15 actions. However, the function of the FP receptor is not fully understood, due in part to significant species differences in the tissue distribution of this receptor (Ocklind *et al.* 1996, *Invest. Ophthalmol. Vis. Sci.* 37: 716-726; Davis & Sharif 1999; Sharif *et al.* 1999).

Griffin *et al.* (*J. Pharmacol. Exp. Ther.* 1999, 290: 1278-1284) reported the  
20 discovery of a selective FP receptor antagonist (AL-8810) of micromolar potency. Sharif *et al.* (*J. Pharm. Pharmacol.* 2000, 52: 1529-1539) describe another analogue of PGF<sub>2α</sub> (AL-3138; Ro-22-6641; 11-deoxy-16-fluoro PGF<sub>2α</sub>) which is a partial agonist of low efficacy and which also functions as an FP receptor antagonist. AL-3138 being a relatively selective agent may be a  
25 valuable FP receptor antagonist tool for investigating the specific function of the FP receptor in various biological systems.

Cyclooxygenase (COX) enzymes, also called prostaglandin endoperoxide synthase (PGHS), catalyse the rate limiting step in the conversion of arachidonic acid to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>). In turn PGH<sub>2</sub> serves as a substrate for specific

prostaglandin synthase enzymes that synthesise the natural prostaglandins. These are named according to the prostaglandin they produce such that prostaglandin D<sub>2</sub> is synthesised by prostaglandin-D-synthase, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by prostaglandin-E-synthase (PGES) and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) by  
5 prostaglandin-F-synthase (PGFS). To date, there are two identified isoforms of the COX enzyme, COX-1 and COX-2 (DeWitt, 1991). COX-1 is constitutively expressed in many tissues and cell types and generates prostaglandins for normal physiological function (Herschman, 1996). By contrast, the expression of COX-2 is rapidly induced following stimulation of quiescent cells by growth  
10 factors, oncogenes, carcinogens and tumour-promoting phorbol esters (Herschman, 1996; Subbaramaiah *et al.*, 1996).

We have shown that expression of the PGF<sub>2α</sub> receptor in the uterus across the menstrual cycle demonstrates higher level of the receptor during the proliferative phase of the endometrium compared with other stages. Expression  
15 in uterine carcinoma tissue is significantly elevated compared with normal uterine tissue. Using an endometrial epithelial cell line, we have demonstrated that PGF<sub>2α</sub> induces proliferation of epithelial cells. This proliferation can be inhibited by using specific inhibitors of the PLC signalling pathway.

These observations demonstrate the possibility of antagonising the PGF<sub>2α</sub> (FP)  
20 receptor to combat menorrhagia.

Antagonists of the FP receptor have been suggested for treating or preventing premature delivery of a foetus and dysmenorrhea, acting by the mechanism of relaxation of smooth muscle (WO 99/32640 and WO 00/17348). They have not, however, been previously suggested to be useful in combating menorrhagia.

25 A first aspect of the invention provides a method of treating or preventing menorrhagia in a female individual, the method comprising administering to the individual at least one agent that prevents PGF<sub>2α</sub> having its effect on the FP receptor.

It is believed that in some cases menorrhagia may be associated with overproliferation of the uterine epithelium.

The female individual may be any individual or patient who is suffering from menorrhagia or a patient who is at risk from menorrhagia. Any premenopausal  
5 or perimenopausal woman is at risk of menorrhagia; however, menorrhagia is more common at the beginning and end of a woman's reproductive life so typically there is a greater risk when a woman's periods first start and in women over 40 years of age.

The patient to be treated may be any female individual who would benefit from  
10 such treatment. Typically and preferably the patient to be treated is a human female. However, the methods of the invention may be used to treat female mammals, such as the females of the following species: cows, horses, pigs, sheep, cats and dogs. Thus, the methods have uses in both human and veterinary medicine.

15 Typically, the agent is one which prevents or disrupts  $\text{PGF}_{2\alpha}$ -mediated signalling of the FP receptor.

Preferably, an agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor prevents or reduces the binding of  $\text{PGF}_{2\alpha}$  to the FP receptor. Alternatively or  
20 additionally, the agent may affect the interaction between  $\text{PGF}_{2\alpha}$  and the FP receptor, or the interaction between the FP receptor and the associated  $G_{\alpha q}$  protein, thus inhibiting or disrupting the  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway.

In one preferred embodiment, the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor may be an antagonist of the FP receptor. FP receptor antagonists  
25 are typically molecules which bind to the FP receptor, compete with the binding of the natural ligand  $\text{PGF}_{2\alpha}$ , and inhibit or disrupt the  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway.

In one preferred embodiment, preventing  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor includes occupying the  $\text{PGF}_{2\alpha}$  binding site on the prostaglandin receptor, such that the natural ligand ( $\text{PGF}_{2\alpha}$ ) is prevented from binding in a mode that would result in its normal mode of signalling via  $\text{Gq/Gq}_\text{H}$  through inositolphosphate and subsequent mobilisation of intracellular calcium.

Alternatively, the receptor antagonist may be a molecule which binds to the FP receptor without preventing  $\text{PGF}_{2\alpha}$  binding thereto, but which disrupts the interaction between  $\text{PGF}_{2\alpha}$  and the FP receptor, thus inhibiting or disrupting  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway.

Further alternatively, the FP receptor antagonist may be a molecule which binds to the FP receptor and which disrupts the interaction between the FP receptor and the associated  $\text{G}_{\alpha\text{q}}$  protein, thus inhibiting or disrupting FP mediated signal transduction pathway.

In an alternative preferred embodiment, the agent may be an antagonist of  $\text{PGF}_{2\alpha}$ .  $\text{PGF}_{2\alpha}$  antagonists are typically molecules which bind to  $\text{PGF}_{2\alpha}$  and prevent or reduce  $\text{PGF}_{2\alpha}$  binding to its receptor, which inhibits or disrupts the  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway. This is the 'soluble receptor' approach in which typically either a part of the receptor or an antibody binds to  $\text{PGF}_{2\alpha}$ .

Alternatively, the  $\text{PGF}_{2\alpha}$  antagonist may be a molecule which binds to  $\text{PGF}_{2\alpha}$  without preventing or reducing the binding of  $\text{PGF}_{2\alpha}$  to the FP receptor, but which disrupts the interaction between  $\text{PGF}_{2\alpha}$  and the FP receptor such that the  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway is inhibited or disrupted. This could be a molecule which binds in a covalent fashion to  $\text{PGF}_{2\alpha}$  and has no effect on binding potency but effects the  $\text{G-protein/IP/Ca}^{2+}$  mechanisms.

In one preferred embodiment, the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor comprises an antagonist of the FP receptor, which may be any



FP receptor antagonist that is suitable to be administered to a patient. The receptor antagonists are typically selective to the particular receptor and preferably have an equal or higher binding affinity to the FP receptor than does  $\text{PGF}_{2\alpha}$ . Although antagonists with a higher affinity for the receptor than the natural ligand are preferred, antagonists with a lower affinity may also be used, but it may be necessary to use these at higher concentrations. Preferably, the antagonists bind reversibly to the FP receptor. Preferably, antagonists are selective for a particular receptor and do not affect other receptors; thus, typically, an FP receptor antagonist binds the FP receptor but does not substantially bind any other receptor.

The peptides listed in Table 1 are reported to be antagonists of the FP receptor that disrupt the interaction between the FP receptor and the associated  $G_{\alpha q}$  protein (WO 99/32640 and WO 00/17438). The amino acids are indicated according to the standard IUPAC single letter convention, and X is cyclohexyl alanine. Lower case letters indicate L-amino acids and capital letters indicate D-amino acids. All of the disclosure in WO 99/32640 and WO 00/17438 relating to specific peptides as FP receptor antagonists, is hereby incorporated herein by reference.

Table 1

PCP-1	RVKFKSQHRQGRSHHLEM
PCP-2	RKAVLKNLYKLASQCCGVHVISLHIWELSSIKNSLKVAAISESP VAEKSAST
PCP-3	CLSEEAKEARRINDEIERQLRRDKRDARRE-NH <sub>2</sub>
PCP-4	KDTILQLNLKEYNLV-NH <sub>2</sub>
PCP-8	ilghrdyk
PCP-10	wedrfyll
PCP-13	ILGHRDYK
PCP-14	YQDRFYLL
PCP-13.7	ILAHRDYK

PCP-13.8	ILaHRDYK
PCP-13.11	ILGFRDYK
PCP-13.13	ILGHKDYK
PCP-13.14	ILGHRNYK
PCP-13.18	ILGHQDYK
PCP-13.20	ILGHRDY-amide
PCP-13.21	ILGHRDYK-amide
PCP-13.22	ILGWRDYK
PCP-13.24	ILGXRDYK
PCP-15	SNVLCsIF

When the antagonist comprises a peptide, such as those mentioned in Table 1, the antagonist may also comprise protein fusions or peptidomimetics thereof.

PGF<sub>2α</sub> dimethyl amide, obtained from Cayman Chemical, Ann Arbor, MI, USA was reported to be a PGF<sub>2α</sub> receptor antagonist (Arnould *et al.*, (2001) *Am. J. Pathol.*, 159(1): 345-357).

AL-8810 ((5Z, 13E)-(9S,11S,15R)-9,15-dihydroxy-11-fluoro-15-(2-indanyl)-16, 17, 18, 19, 20-pentanor-5,13-prostadienoic acid) obtained from Alcon Research was reported to be a weak partial agonist of the PGF<sub>2α</sub> receptor and a highly selective antagonist of the PGF<sub>2α</sub> receptor. AL-8810 was reported not to significantly inhibit functional responses of prostaglandin receptors TP, DP, EP2 or EP4 at high 10 μM concentration (Griffin *et al.*, (1999) *J. Pharmacol. Exp. Ther.*, 260(3): 1278-1284).

AL-3138 (11-deoxy-16-fluoro PGF<sub>2α</sub>) was reported to be a weak partial agonist of the PGF<sub>2α</sub> receptor, and also a highly selective antagonist of the PGF<sub>2α</sub> receptor (Sharif *et al.*, (2000) *J. Pharm. Pharmacol.*, 52(12): 1529-1539).

Phloretin was reported to be a PGF<sub>2α</sub> receptor antagonist (Kitanaka *et al* (1993) *J. Neurochem.* 60(2): 704-708).

The sulfonylhurea glibenclamide was reported to be a  $\text{PGF}_{2\alpha}$  receptor antagonist (Delacy and Van de Voorde (1995), *Eur. J. Pharmacol.* 280(2): 179-184). The sulfonylhureas tolbutamide and tolazamide were reported to be very weak antagonists of the FP receptor. (Sharif et al (2000) *J. Pharm. Pharmacol.*,  
5 52(12): 1529-1539).

$\text{PGF}_{2\alpha}$  dimethyl amine was reported to be a  $\text{PGF}_{2\alpha}$  receptor antagonist (Stinger et al (1992), *J. Pharmacol. Exp. Ther.*, 220: 521-525).

(E)-5-[[[(3-pyridinyl)[3-(trifluoromethyl) phenyl]methylen]amino]oxy]pentanoic acid, also known as ridogrel, obtained from Janssen Pharmaceutica, was  
10 reported to be a  $\text{PGF}_{2\alpha}$  receptor antagonist (Janssens et al., (1990), *Thrombosis and Haemostasis*, 64(1): 91-96).

The compound PHG113 was reported to be a selective  $\text{PGF}_{2\alpha}$  receptor antagonist (Quiniou et al., (2001) *Pediatric Research*, 49(2): 452A).

EP-128479 describes pyrazolyl-methyl-ergoline derivatives which are reported  
15 to be  $\text{PGF}_{2\alpha}$  receptor antagonists. All the disclosure in EP-128479 relating to pyrazolyl-methyl-ergoline derivatives as  $\text{PGF}_{2\alpha}$  receptor inhibitors, is hereby incorporated herein by reference.

In a further preferred embodiment of the invention, the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor may be an antagonist of  $\text{PGF}_{2\alpha}$ , which  
20 may be any  $\text{PGF}_{2\alpha}$  antagonist that is suitable to be administered to the patient. The  $\text{PGF}_{2\alpha}$  antagonists are preferably selective to  $\text{PGF}_{2\alpha}$  and typically have a higher binding affinity for  $\text{PGF}_{2\alpha}$  than for other molecules. Although antagonists with a higher affinity for  $\text{PGF}_{2\alpha}$  than other molecules are preferred, antagonists with a lower affinity may also be used, but it may be necessary to  
25 use these at higher concentrations. Preferably, the  $\text{PGF}_{2\alpha}$  antagonists bind reversibly to  $\text{PGF}_{2\alpha}$ .

PGF<sub>2α</sub> antagonists include anti-PGF<sub>2α</sub> antibodies such as rabbit polyclonal anti-PGF<sub>2α</sub> antibodies from Oxford Biomedical Research, Inc., Oxford, UK (Arnould *et al.*, *Am. J. Pathol.* 2001 159(1): 345-357). Arnould *et al* state that, according to the manufacturer, the specificity of the antibody is very high and the cross-reactivity with other prostanoid derivatives is <1%.

JP 04077480; JP 08176134; JP 01199958; JP 01050818; and JP 63083081 each describe phthalide derivatives that are reported to be PGF<sub>2α</sub> inhibitors. All the disclosure in JP 04077480; JP 08176134; JP 01199958; JP 01050818; and JP 63083081 relating to phthalide derivatives as PGF<sub>2α</sub> inhibitors, is hereby incorporated herein by reference.

WO 91/13875 describes (iso) quinoline sulphonamide compounds which are reported to be PGF<sub>2α</sub> inhibitors. All the disclosure in WO 91/13875 relating to (iso) quinoline sulphonamide compounds as PGF<sub>2α</sub> inhibitors, is hereby incorporated herein by reference.

Some of the compounds reported as being inhibitors or antagonists of PGF<sub>2α</sub> may, in fact, be antagonists of the PGF<sub>2α</sub> (FP) receptor, as used and defined herein. References to such compounds as inhibitors or antagonists of PGF<sub>2α</sub> should therefore be considered as references to FP receptor antagonists.

As used herein, the term 'antagonist' covers all types of antagonism. GPCRs such as prostaglandin receptors are known to show inverse agonism which has the outcome of blocking a desired response. Thus a suitable FP antagonist for use in the present invention may be identified by measuring the binding of a radio-labelled FP agonist to PGF<sub>2α</sub> with or without the purported antagonist. Secondly, FP antagonists may be identified in a functional assay *eg* by showing that the effect of an FP agonist on Ca<sup>2+</sup> levels is modified in the presence of the antagonist. Thirdly FP antagonists may be identified by inhibition of epithelial cell growth in cell culture.

All of the patents and other documents referred to herein, and in particular those describing antagonists or inhibitors of FP receptors and of  $\text{PGF}_{2\alpha}$ , are incorporated herein, in their entirety, by reference.

It will be appreciated that one or more agents that prevent  $\text{PGF}_{2\alpha}$  having its  
5 effect on the FP receptor may be administered to the patient. These may all be considered "treatment agents" of the invention. It will also be appreciated that when more than one treatment agent is administered to the patient, they may be administered sequentially or in combination.

The treatment agent(s) are administered in an effective amount to combat the  
10 menorrhagia. Thus, the treatment agents may be used to alleviate symptoms (ie are used palliatively), or may be used to treat the condition, or may be used prophylactically to prevent the condition. The treatment agent may be administered by any suitable route, and in any suitable form.

Typically, the aforementioned treatment agents for use in the invention are  
15 administered in a quantity and frequency such that an effective dose is delivered to at least 90% of the FP receptors ( $\text{ED}_{90}$ ). The potency of the molecule would dictate the dose, as would the formulation and route of administration.

The aforementioned treatment agents for use in the invention or a formulation thereof may be administered by any conventional method including oral and  
20 parenteral (eg subcutaneous or intramuscular) injection. The treatment may consist of a single dose or a plurality of doses over a period of time. The dose to be administered is determined upon consideration of age, body weight, mode of administration, duration of the treatment and pharmacokinetic and toxicological properties of the treatment agent or agents. The treatment agents are  
25 administered at a dose (or in multiple doses) which produces a beneficial therapeutic effect in the patient. Typically, the treatment agents are administered at a dose the same as or similar to that used when the treatment agent is used for

another medical indication. In any event, the dose suitable for treatment of a patient may be determined by the physician.

Whilst it is possible for a treatment agent of the invention to be administered alone or in combination with other said treatment agents, it is preferable to  
5 present it or them as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the treatment agent of the invention and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free.

10 The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the treatment agent or agents with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the  
15 active ingredient (ie treatment agent or agents) with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a  
20 powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more  
25 accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant

(eg sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or  
5 scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or  
10 tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier. Buccal administration is also preferred.

Formulations suitable for parenteral administration include aqueous and non-  
15 aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and  
20 vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily  
25 sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in

the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Certain of the treatment agents are proteins or peptides. Proteins and peptides may be delivered using an injectable sustained-release drug delivery system.

- 5 These are designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period.

- 10 The protein and peptide can be administered by a surgically implanted device that releases the drug directly to the required site. For example, Vitrasert releases ganciclovir directly into the eye to treat CMV retinitis. The direct application of this toxic agent to the site of disease achieves effective therapy without the drug's significant systemic side-effects.

- 15 Electroporation therapy (EPT) systems can also be employed for the administration of proteins and peptides. A device which delivers a pulsed electric field to cells increases the permeability of the cell membranes to the drug, resulting in a significant enhancement of intracellular drug delivery.

- 20 Proteins and peptides can be delivered by electroincorporation (EI). EI occurs when small particles of up to 30 microns in diameter on the surface of the skin experience electrical pulses identical or similar to those used in electroporation. In EI, these particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

- 25 An alternative method of protein and peptide delivery is the ReGel injectable system that is thermo-sensitive. Below body temperature, ReGel is an injectable liquid while at body temperature it immediately forms a gel reservoir that slowly



erodes and dissolves into known, safe, biodegradable polymers. The treatment agent is delivered over time as the biopolymers dissolve.

Protein and peptide pharmaceuticals can also be delivered orally. The process employs a natural process for oral uptake of vitamin B<sub>12</sub> in the body to co-deliver proteins and peptides. By riding the vitamin B<sub>12</sub> uptake system, the protein or peptide can move through the intestinal wall. Complexes are synthesised between vitamin B<sub>12</sub> analogues and the drug that retain both significant affinity for intrinsic factor (IF) in the vitamin B<sub>12</sub> portion of the complex and significant bioactivity of the drug portion of the complex.

- 10 Proteins and polypeptides can be introduced to cells by "Trojan peptides". These are a class of polypeptides called penetratins which have translocating properties and are capable of carrying hydrophilic compounds across the plasma membrane. This system allows direct targeting of oligopeptides to the cytoplasm and nucleus, and may be non-cell type specific and highly efficient.
- 15 See Derossi *et al* (1998), *Trends Cell Biol* 8, 84-87.

The treatment agents or formulations may also be administered transdermally, *eg* as a patch, gel, lotion, cream or oil.

It is preferred if the treatment agent (or agents) is administered orally.

- It is further preferred if the treatment agent (or agents) is administered to the female reproductive system. For example, the treatment agent or agents may suitably be administered intravaginally using, for example, a gel or cream or vaginal ring or tampon. The treatment agent may also advantageously be administered by intrauterine delivery, for example using methods well known in the art such as an intrauterine device.

- 25 Typically, the gel or cream is one which is formulated for administration to the vagina. It may be oil based or water based. Typically, the treatment agent (or

agents) is present in the cream or gel in a sufficient concentration so that an effective amount is administered in a single (or in repeated) application.

Typically, the vaginal ring comprises a polymer which formed into a "doughnut" shape which fits within the vagina. The treatment agent (or agents) is present within the polymer, typically as a core, which may dissipate through the polymer and into the vagina and/or cervix in a controlled fashion. Vaginal rings are known in the art. The vaginal ring may be disposable and is retained intravaginally during the woman's period and therefore contains sufficient of the treatment agent(s) to be released and to be effective during the woman's period. Alternatively, the vaginal ring may be used over a time interval of around three months to one year, during which time sufficient of the treatment agent(s) is released to have a beneficial effect over that period of time. It will be appreciated that the polymer from which the ring is made, the size and shape of the ring and the content of the treatment agent, as well as other parameters, may be selected by reference to whether the ring is for use in one cycle or for longer spells.

Typically, the tampon is impregnated with the treatment agent (or agents) and that a sufficient amount of the treatment agent (or agents) is present in the tampon.

Typically, the intrauterine device is for placing in the uterus over extended periods of time, such as between one and five years. Typically, the intrauterine device comprises a plastic frame, often in the shape of a "T" and contains sufficient of the treatment agent(s) to be released over the period of use. The agent is generally present within or encompassed by a slow-release polymer which forms part of the device, such as in the form of a "sausage" of agent which wraps around the long arm of the "T" which is typically covered with a controlled-release membrane. Intrauterine devices are known in the art.

The invention also provides combinations (such as in a pharmaceutical formulation) of one or more of the treatment agents as described herein, and one or more agents presently used to treat menorrhagia, such as tranexamic acid or mefenamic acid.

- 5 A second aspect of the invention provides use of at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor, in the manufacture of a medicament for treating or preventing menorrhagia.

A third aspect of the invention provides a pharmaceutical composition comprising at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP  
10 receptor, for treating or preventing menorrhagia.

Preferably, in the second and third aspects of the invention, the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor is as defined with respect to the first aspect of the invention.

The invention will now be described in more detail by reference to the following  
15 Figures and Examples.

#### Figure 1

*In situ* hybridisation for FP receptor in (a-f) normal human endometrium, and in (g-i) adenocarcinoma tissues.

#### Figure 2

- 20 Quantitative RT-PCR expression of FP receptor in a) normal human endometrium and b) adenocarcinoma tissues ( $p < 0.05$ ). In a), EP is early proliferative phase, MP/LP is mid/late proliferative phase, ES is early secretory phase and LS is late secretory phase. In b), "Cycle" is an average of EP, MP/LP, ES and LS from a).

#### 25 Figure 3

IP3 production in human endometrium.

**Figure 4**

ERK phosphorylation in Ishikawa cells.  $\text{PGF}_{2\alpha}$  100nM was incubated for 10 and 30 min. U73122 2 $\mu$ M was pre-incubated for 60 min before treatment with  $\text{PGF}_{2\alpha}$ .

**5 Figure 5**

Proliferation in Ishikawa cells following  $\text{PGF}_{2\alpha}$ . Cells were treated overnight with  $\text{PGF}_{2\alpha}$ . All inhibitors were added for 60 min before the addition of  $\text{PGF}_{2\alpha}$  ( $n \geq 4$ ;  $p < 0.05$ ).

**Example 1: Prostaglandin (PG)  $\text{F}_{2\alpha}$  receptor expression and localisation in**  
**10 human endometrium and endometrial adenocarcinoma: role of  $\text{PGF}_{2\alpha}$  in**  
**epithelial cell proliferation**

*Summary*

$\text{PGF}_{2\alpha}$  is one of the prostaglandins generated by human endometrium and can be measured in menstrual fluid and from endometrial explants cultured *in vitro*.  
**15**  $\text{PGF}_{2\alpha}$  production is stimulated by oestrogen, which induces an up-regulation of COX expression, and inhibited by progesterone, which decreases COX expression and increases PGDH expression. Increased COX expression has been demonstrated in a number of different cancers and over-expression in rat intestinal epithelial (RIE) cells induces an altered cell type with increased  
**20** proliferation and invasiveness *in vivo*. The aims of this study were to identify the target cells for  $\text{PGF}_{2\alpha}$  in human endometrium and endometrial adenocarcinoma and quantify FP receptor expression in these tissues. The role of  $\text{PGF}_{2\alpha}$  in epithelial cell proliferation, and the specific signalling pathways involved, was then determined in an endometrial adenocarcinoma cell line  
**25** (Ishikawa). We observed a significantly increased expression of FP mRNA in mid- to late-proliferative tissue that was further increased in endometrial adenocarcinomas. Localisation of FP mRNA was identified in epithelial cells

from only mid- and late-proliferative tissue samples. In uterine adenocarcinoma tissue, FP expression was consistently localised in epithelial cells and was independent of differentiation stage. Negligible FP mRNA expression was seen in adjacent stromal cells. Inositol mobilisation in response to  $\text{PGF}_{2\alpha}$  was  
5 determined in normal endometrium and endometrial adenocarcinoma and was significantly increased in all samples following  $\text{PGF}_{2\alpha}$ , however, no additional inositol mobilisation was found in adenocarcinoma samples. In Ishikawa cells,  $\text{PGF}_{2\alpha}$  also induced inositol mobilisation and produced a concentration-dependent increase in cell proliferation that was inhibited PLC-dependent but  
10 not affected by either p38 or p42/p44 MAPK inhibitors. These results demonstrate that proliferating endometrial epithelial cells are responsive to  $\text{PGF}_{2\alpha}$  and indicate a role for  $\text{PGF}_{2\alpha}$  in menorrhagia.

### *Introduction*

Prostaglandin (PG)  $\text{F}_{2\alpha}$  is a prostanoid belonging to the eicosanoid family of  
15 biologically active lipid (1). Other members of this prostanoid family include  $\text{PGD}_2$ ,  $\text{PGE}_2$ , prostacyclin ( $\text{PGI}_2$ ) and thromboxane  $\text{A}_2$  ( $\text{TxA}_2$ ) that are all synthesised from arachidonic acid by a combination of cyclooxygenase (COX) and specific synthase enzymes (2). To-date, there are two identified isoforms of the COX enzyme; constitutively expressed COX-1 that generates PG for normal  
20 physiological function and COX-2, an early response gene whose expression can be rapidly induced (3). COX metabolises arachidonic acid to the unstable intermediate  $\text{PGH}_2$  and specific synthase enzymes then convert  $\text{PGH}_2$  to the PG molecules (4-8). The PG synthase enzymes are named according to the prostaglandin they produce such that  $\text{PGF}_{2\alpha}$  is a metabolite of prostaglandin-F-synthase. Once synthesised PG mediate their actions via seven-transmembrane  
25 G-protein coupled receptors (GPCR) specific to each prostanoid.  $\text{PGF}_{2\alpha}$  receptor (FP) has been cloned in humans and transduces its response via the G-protein  $\text{G}_q$ , PLC activation and generation of inositol-trisphosphate that in turn mobilises intracellular  $\text{Ca}^{2+}$ .

COX enzymes and PG have recently been demonstrated to regulate epithelial cell growth and angiogenesis. In rat intestinal epithelial cells, COX-2 expression and PGE<sub>2</sub> synthesis are associated with increased cellular proliferation and resistance to apoptosis (9,10). The same group also showed that expression of  
5 COX-2 in epithelial cells enhances the expression of angiogenic factors that act in a paracrine manner to induce endothelial cell migration and microvascular tube formation (9). In human endometrium, COX-2 enzyme expression is maximal during the proliferative phase and is localised to epithelial and perivascular cells (10-14).

10 PGF<sub>2α</sub> is also a major metabolite of COX in human endometrium and as well as being present in menstrual fluid is released by human endometrial explants in culture (15). However, due to the potent luteolytic activity of PGF<sub>2α</sub> and the demonstrated increase in myometrial contractility following PGF<sub>2α</sub>, most studies have focused on FP receptor expression and regulation in the ovary. FP receptor  
15 expression and the role of PGF<sub>2α</sub> within the functionalis layer of human endometrium have not been fully examined.

Original studies identified in separated bovine endometrial cells cultured *in vitro*, that epithelial cells preferentially release PGF<sub>2α</sub> in contrast to stromal cells that secrete predominately PGE<sub>2</sub> (16). Moreover, studies measuring FP mRNA  
20 in non-primate species demonstrated increased FP receptor expression with oestrogen where as progesterone decreased FP receptor expression when animal were ovariectomised (17-19).

The aims of this study were to localise and quantify FP receptor expression both in human endometrium and in differing grades of human uterine  
25 adenocarcinoma to identify the PGF<sub>2α</sub>-responsive cells. Mobilisation of inositol phosphates and phosphorylation of the MAPK extracellular-regulated kinase (ERK) were used to determine the presence of functional FP receptors and BrdU incorporation in Ishikawa cells as an in-vitro proliferation assay. The data from

this study demonstrate epithelial expression of FP in only proliferating tissue with substantially increased FP mRNA and clear epithelial cell localisation in endometrial adenocarcinomas. Moreover,  $\text{PGF}_{2\alpha}$  treatment of Ishikawa cells caused inositol mobilisation, ERK phosphorylation and concentration-dependent increases in cell proliferation.

The data reported herein is the first report of FP expression in human endometrium and demonstrates a potential role of  $\text{PGF}_{2\alpha}$  in uterine epithelial cell proliferation and in menorrhagia.

### Methods

#### 10 Patients and tissue collection

Endometrial biopsies ( $n=12$ ) at different stages of the menstrual cycle were collected with an endometrial suction curette (Pipelle, Laboratoire CCD, France) from women with regular menstrual cycles (25-35 days). In addition, full thickness endometrial biopsies ( $n=18$ ) at all stages of the menstrual cycle ( $n=3$  from early, mid and late proliferative and  $n=3$  from early mid and late secretory) were collected from women undergoing hysterectomy for benign gynaecological indications. Shortly after pipelle suction or hysterectomy, tissue was either snap frozen in dry ice and stored at  $-70^{\circ}\text{C}$  (for RNA extraction), fixed in neutral buffered formalin (NBF) and wax embedded (for in-situ hybridisation studies), or placed in RPMI 1640 (containing 2 mmol/l L-glutamine, 100 U penicillin and 100  $\mu\text{g/ml}$  streptomycin) and transported to the laboratory for *in vitro* culture. All subjects reported regular menstrual cycles (cycle length 25-35 days) and no women had received a hormonal preparation in the 3 months preceding biopsy collection. Biopsies were dated according to stated last menstrual period (LMP) and confirmed by histological assessment according to criteria of Noyes and co-workers (20). Furthermore, circulating oestradiol and progesterone concentrations at the time of biopsy were consistent for both stated LMP and histological assignment of menstrual cycle stage. Ethical approval was obtained

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from Lothian Research Ethics Committee and written informed consent was obtained from all subjects before tissue collection.

*In situ hybridisation (ISH)*

Custom synthesis oligonucleotide double FITC-labelled cDNA probes for FP  
5 receptor were obtained from Biognostik GmbH (Germany). Sections (5 µm)  
were cut onto Gelatin coated Superfrost slides (BDH Laboratory Supplies, UK)  
from full thickness human uterine biopsies collected across the menstrual cycle  
(n=18). Tissue was dewaxed in xylene, rehydrated using increasing  
concentrations of ethanol before Proteinase K treatment (100 µg/ml in Tris-HCl  
10 pH 7.6 100 mM containing EDTA 50 mM) for 15 min at 37°C to enhance cDNA  
probe access. After washing in DEPC-H<sub>2</sub>O, hybridisation mixture (50 µl;  
supplied with probe) was added to each section and slides incubated for 4 h at  
30°C before adding cDNA probe (6U/ml hybridisation mix) and incubating  
overnight at 30°C. Post-hybridisation washes of 1x SSC for 5 min (twice) and  
15 0.1x SSC at 42°C for 15 min (twice) were completed before detecting the FITC-  
labelled probe using standard ICC reagents (TSA Biotin System, NEN Life  
Sciences, UK). Endogenous peroxidase activity was first blocked with 3% H<sub>2</sub>O<sub>2</sub>  
in methanol for 30 min before incubating sections with blocking buffer for 30  
min. Conjugated anti-FITC-HRP (Boehringer-Mannheim, Check) was added in  
20 blocking buffer and the sections incubated for 60 min. After washing, biotiny  
l tyramide amplification reagent was applied to each slide and incubated for 15  
min. Streptavidin-HRP was applied after washing and incubated for 30 min and  
probe localisation visualised with DAB. Control oligonucleotide double FITC-  
labelled cDNA probe containing the same proportion of cysteine (C) and  
25 guanine (G) bases as the FP receptor probe was included to assess background  
hybridisation. All treatments were carried out at room temperature unless  
otherwise specified.

*Taqman quantitative RT-PCR*

Endometrial RNA samples were extracted from endometrial biopsies (n=33) using Tri-reagent (Sigma, UK) following the manufacturer's guidelines. Once extracted and quantified, RNA samples were reverse transcribed using MgCl<sub>2</sub> (5.5 mM), dNTPs (0.5 mM each), random hexamers (2.5 µM), RNAase inhibitor (0.4 U/µl) and multiscribe reverse transcriptase (1.25 U/µl; all from PE Biosystems, Warrington, UK). The mix was aliquoted into individual tubes (16 µl/tube) and template RNA was added (4 µl/tube of 100 ng/µl RNA). After mixing by brief centrifugation, samples were incubated for 90 minutes at 25°C, 45 minutes at 48°C and 95°C for 5 minutes. Thereafter cDNA samples were stored at -20°C. A tube with no reverse transcriptase was included to control for any DNA contamination.

To measure cDNA expression a reaction mix was prepared containing Taqman buffer (5.5 mM MgCl<sub>2</sub>, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 400 µM dUTP), ribosomal 18S forward and reverse primers and probe (all at 50 nM), forward and reverse primers for EP receptor (300 nM), EP receptor probe (100 nM), AmpErase UNG (0.01 U/µl) and AmpliTaq Gold DNA Polymerase (0.025 U/µl; all from PE Biosystems). After mixing, 48 µl was aliquoted into separate tubes and 2 µl/replicate (40 ng) of cDNA added and mixed before placing duplicate 24 µl samples into a PCR plate. A no template control (containing water) was included in triplicate. Wells were sealed with optical caps and the PCR reaction carried out using an ABI Prism 7700. FP receptor primers and probe for quantitative PCR were designed using the PRIMER express program (PE Biosystems). The sequence of the FP receptor primers and probe were; Forward 5'-GCA GCT GCG CTT CTT TCA A-3'; Reverse 5'-CAC TGT CAT GAA GAT TAC TGA AAA AAA TAC-3'; Probe (FAM labelled) 5'-CAC AAC CTG CCA GAC GGA AAA CCG-3'.

The ribosomal 18S primers and probe sequences were; Forward 5' -CGG CTA CCA CAT CCA AGG AA-3'; Reverse 5'-GCT GGA ATT ACC GCG GCT-3';

Probe (VIC labelled) 5'-TGC TGG CAC CAG ACT TGC CCT C-3'. Data were analysed and processed using Sequence Detector v1.6.3 (PE Biosystems) as instructed by the manufacturer. Briefly, the software calculates the reaction cycle number at which fluorescence reaches a determined level for both 18S control and FP receptor. This is the relative abundance of FP receptor in each sample and by comparing to an internal positive control, relative expression can be determined. Results are expressed as relative expression to the internal positive standard.

#### *Total Inositol Phosphate (InsP) Assays*

PGF<sub>2α</sub> stimulation of total InsP production was as described (21). Briefly, tissue samples or Ishikawa cells were incubated with inositol free DMEM containing 1% dialyzed heat-inactivated FCS and 0.5 µCi/well myo-[<sup>3</sup>H]inositol (Amersham Pharmacia Biotech) for 48 hours. Medium was removed, and cells washed with 1 ml buffer (140 mM NaCl, 20 mM HEPES, 4 mM KCl, 8 mM glucose, 1 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 1 mg/ml bovine serum albumin) containing 10 mM LiCl. Cells were then incubated for 1 hour at 37°C in 1 ml buffer with or without inhibitors. Following incubation agonist was added at the required concentration and cells incubated for 1 hour. Reactions were terminated by the removal of agonist and the addition of 500 µl ice cold 10 mM formic acid, which was incubated for 30 minutes at 4°C. Total [<sup>3</sup>H] inositol phosphates was separated from the formic acid cell extracts on AG 1-X8 anion exchange resin (Bio-Rad) and eluted with a 1 M ammonium formate/0.1 M formic acid solution. The associated radioactivity was determined by liquid scintillation counting and plotted relative to protein concentrations determined using the modified Lowry method.

#### *Proliferation assay*

Proliferation of Ishikawa cells was determined using a BrdU incorporation ELISA (Roche Diagnostics GmbH, Mannheim, Germany). Briefly, Ishikawa

cells were seeded at  $5 \times 10^3$  cells per well in a 96-well plate and allowed to adhere overnight. Cells were next starved for 24 hr with indomethacin  $1.5 \mu\text{g/ml}$  before 24 h  $\text{PGF}_{2\alpha}$  treatment ( $1\text{nM} - 1\mu\text{M}$ ) in serum-free medium containing indomethacin. Inhibitors were added to cells for 60 min before  $\text{PGF}_{2\alpha}$  with the control well receiving the same concentration of vehicle. Following 24 h treatment, cells were labelled with BrdU for 4 h, then fixed and assayed for BrdU incorporation using standard immunohistochemical techniques. Results were plotted as percentages of untreated cells.

#### Statistics

- Where appropriate, data were subjected to statistical analysis with ANOVA and Fishers PLSD tests (Statview 4.0; Abacus Concepts Inc., USA) and statistical significance accepted when  $p < 0.05$ .

#### Results

FP receptor expression in human endometrium demonstrated a distinctive localisation pattern across the cycle. FP receptor localised to glandular epithelial cells in only mid- and late-proliferative stages of the menstrual cycle and was absent in all other biopsies examined. Only occasional expression was observed in perivascular cells and was independent of the cycle stage. In uterine adenocarcinoma biopsies FP receptor expression was also localised to epithelial cells. Epithelial cell expression of FP receptor was observed in all differentiation types with no discernible change in pattern between poor, moderately or well differentiated samples.

Quantification of FP receptor mRNA expression in both endometrial and adenocarcinoma samples was determined by Taqman quantitative RT-PCR. A significant increase in relative FP receptor expression was observed in mid- to late-proliferative endometrium ( $0.40 \pm 0.02$ ;  $p \leq 0.05$ ) when compared to early proliferative ( $0.06 \pm 0.02$ ) and all secretory phases of the menstrual cycle ( $0.07 \pm$

0.01). Within human adenocarcinoma samples relative FP receptor mRNA expression was significantly increased ( $116.3 \pm 63.6$ ) compared to cycle endometrium ( $0.15 \pm 0.04$ ). No correlation was observed between the different grades of adenocarcinoma, however, a large variance was observed within these tissues.

Human endometrium produced a concentration-dependent increase in total InsP production following  $\text{PGF}_{2\alpha}$  treatment. Maximal InsP mobilisation was observed with  $\text{PGF}_{2\alpha}$  100nM and inhibited by pre-treatment with U73122 2 $\mu\text{M}$ , an inhibitor of PLC. Total inositol phosphate production was also measured in Ishikawa cells following treatment with  $\text{PGF}_{2\alpha}$ .  $\text{PGF}_{2\alpha}$  produced a concentration dependent increase in total InsP with  $\text{PGF}_{2\alpha}$  100nM producing a maximum of 29 cpm/mg protein.

Treatment of Ishikawa cells with  $\text{PGF}_{2\alpha}$  100nM induced phosphorylation of extracellular regulated kinase (ERK) in Ishikawa cells. Treatment for 10 min with  $\text{PGF}_{2\alpha}$  100 nM induced  $6.6 \pm 0.7$  fold increase in phosphorylated ERK intensity compared to native ERK.

The proliferative effect of  $\text{PGF}_{2\alpha}$  in Ishikawa cells was determined by measuring incorporation of BrdU.  $\text{PGF}_{2\alpha}$  produced a concentration-dependent increase in BrdU incorporation that was maximal at 100 nM ( $136.3 \pm 7.48\%$ ). Pre-treatment of cells with an ERK1/2 inhibitor (PD98059 50 $\mu\text{M}$ ) produced a reduction in basal proliferation ( $84.0 \pm 5.8\%$ ) although  $\text{PGF}_{2\alpha}$  still produced a concentration-dependent increase in proliferation in the presence of PD98059 50 $\mu\text{M}$  (100nM  $\text{PGF}_{2\alpha}$ -induced  $119.4 \pm 11.2\%$  control BrdU incorporation). Pre-incubation with an inhibitor of PLCB (U73122 2 $\mu\text{M}$ ) also produced a slight reduction in basal proliferation ( $93.9 \pm 1.9\%$ ), and in addition, inhibited the concentration-dependent increase in proliferation following  $\text{PGF}_{2\alpha}$  ( $106.1 \pm 6.6\%$  BrdU incorporation by 100nM  $\text{PGF}_{2\alpha}$ ).

*Discussion*

We have demonstrated increased expression of FP receptor in epithelial cells from proliferative endometrium and different grades of adenocarcinoma. Moreover, we have quantified and demonstrated functional FP receptor expression in Ishikawa cells of human endometrial adenocarcinoma origin. In Ishikawa cells,  $\text{PGF}_{2\alpha}$  induced  $\text{IP}_3$  production and phosphorylation of p42/p44 MAPK (ERK). Additionally, following overnight incubation with  $\text{PGF}_{2\alpha}$ , Ishikawa cells demonstrated increased cell proliferation that was inhibited by PLC blockade.

10  $\text{PGF}_{2\alpha}$  has not previously been demonstrated to have a role in cell proliferation of mammalian tissues. Studies to identify whether  $\text{PGF}_{2\alpha}$  was involved in colon adenocarcinomas demonstrated a lack of proliferation by  $\text{PGF}_{2\alpha}$  in HCT-8 and HT-29 human colon adenocarcinoma cell lines. FP receptor knockout mice do not demonstrate any abnormalities in endometrial physiology but instead  
15 demonstrate failures in myometrial function with females being unable to deliver pups at term (22).

The data disclosed herein is the first demonstration of the proliferative effects of  $\text{PGF}_{2\alpha}$  in human endometrial epithelial cells. Maximal FP receptor expression is observed in mid-late proliferative endometrium when oestrogen levels are  
20 elevated.

Without being bound by theory, it is believed that the high FP receptor expression observed in adenocarcinoma samples could relate to levels of progesterone receptors where the ratio of  $\text{PR}_A$ :  $\text{PR}_B$  may be important in endometrial cancer (23). In reproductive tissue carcinomas, such as breast, endometrium and ovary,  
25 oestrogens promote cell proliferation and are implicated in disease progression (24). In the endometrium, progesterone opposes the proliferative effect of oestrogen by promoting cell differentiation and progestin treatment has proved beneficial in the management of endometrial cancer (23, 25). However,



therapeutic benefit is observed only when these tumours express functional progesterone receptors (PR) (25) and combining progestins with oestrogen in hormone replacement therapy (HRT), to increase PR expression, has been found to decrease the risk of endometrial cancer (26, 27). As such, loss of  
5 responsiveness to progesterone could result in loss of progesterone inhibition and explain the observed increase in FP receptor expression observed in uterine adenocarcinoma.

Ishikawa, an endometrial epithelial cell, expresses greater levels of FP receptor than normal endometrium and is responsive to  $\text{PGF}_{2\alpha}$ . We observed  
10 mobilisation of InsP and phosphorylation of ERK in these cells following  $\text{PGF}_{2\alpha}$  as has been previously identified (28, 29). Moreover,  $\text{PGF}_{2\alpha}$  induced increased proliferation in Ishikawa cells that was sensitive to PLC blockade. This is the first documented report showing epithelial cell proliferation in response to  $\text{PGF}_{2\alpha}$ . These observations support the role of  $\text{PGF}_{2\alpha}$  in normal epithelial cell  
15 proliferation and endometrial adenocarcinoma where FP receptor expression is increased, and in menorrhagia.

In summary, this is the first publication to demonstrate FP receptor expression and localisation with human endometrial tissue and human endometrial adenocarcinomas. We have demonstrated epithelial cell expression for FP  
20 receptor and have also demonstrated increased proliferation in endometrial epithelial cells, Ishikawa, following  $\text{PGF}_{2\alpha}$ . These studies clearly indicate a role for  $\text{PGF}_{2\alpha}$  in epithelial cell proliferation. These studies further suggest that  $\text{PGF}_{2\alpha}$  has a role in menorrhagia, and that an agent which prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor, could be used to combat menorrhagia.

25 **Example 2: Expression of FP receptors in uterine tissue of women with menorrhagia compared to women with no menorrhagia**

Uterine tissue is collected by biopsy from women with known indication of menorrhagia and women who have normal uterine function. The tissue is

assessed for the expression of FP receptors. This is assessed using various molecular techniques. The signalling of these receptors in response to  $\text{PGF}_{2\alpha}$  is assessed. Tissue is cultured for various time in the presence or absence of  $\text{PGF}_{2\alpha}$  and the second messenger cAMP is measured in response to these treatments.

- 5 Expression of FP receptors is elevated in the uterine tissue that comes from women with a known history with menorrhagia. Moreover, the signalling events in response to  $\text{PGF}_{2\alpha}$  is augmented in these patients.

Hence in women with menorrhagia, it should prove beneficial to treat with agents that prevent  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor, such as FP  
10 receptor antagonists, in order to block the signalling pathway and ultimately transcription of target genes that may mediate vascular function/dysfunction and excessive bleeding.

#### Example 3: Treatment of menorrhagia with an FP receptor antagonist.

- A woman presents to her physician with symptoms of menorrhagia. The  
15 physician diagnoses menorrhagia. She is administered AL-3138 or AL-8810 at a dosing quantity and frequency such that the therapeutic level of active agent at the site of treatment is maintained at a level ideally EC90 but preferably not less than EC50 throughout the treatment period. The treatment is delivered orally or more locally depending on patient acceptability, avoidance of side effects and  
20 systemic bioavailability.

#### Example 4: Suppository

	<u>mg/suppository</u>
AL-3138 or AL-8821 (63 $\mu\text{m}$ )*	250
Hard Fat, BP (Witepsol H15 - Dynamit Nobel)	<u>1770</u>
25	2020

\*The antagonist AL-3138 or AL-8821 is typically used as a powder wherein at least 90% of the particles are of 63  $\mu\text{m}$  diameter or less.

One fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200 µm sieve and added to the molten base with mixing, using a silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a homogenous mix. The entire suspension is passed through a 250 µm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C 2.02 g of the mixture is filled into suitable plastic moulds. The suppositories are allowed to cool to room temperature.

10 **Example 5: Pessaries**

	mg/pessary
AL-3138 or AL-8821	250
Anhydrate Dextrose	380
Potato Starch	363
15 Magnesium Stearate	<u>7</u>
	1000

The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

**Example 6: Vaginal ring**

- 20 A vaginal ring containing AL-3138 or AL-8821 is produced using core extrusion technology.

**Example 7: Intrauterine device**

An intrauterine device containing AL-3138 or AL-8821 is produced using standard technology.

25 **Example 8: Tampon**

A tampon for treating menorrhagia is produced by impregnating a standard tampon with an effective dose of AL-3138 or AL-8821.

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CLAIMS

1. A method of treating or preventing menorrhagia in a female individual, the method comprising administering to the individual at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor.
2. A method according to Claim 1 wherein the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor prevents or reduces the binding of  $\text{PGF}_{2\alpha}$  to the FP receptor.
3. A method according to Claim 1 or 2 wherein the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor affects the interaction between  $\text{PGF}_{2\alpha}$  and the FP receptor, or the interaction between the FP receptor and the associated  $G_{\alpha q}$  protein, thus inhibiting or disrupting a  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway.
4. A method according to any one of Claims 1 to 3 wherein the agent is an antagonist of the FP receptor.
5. A method according to Claim 4 wherein the FP receptor antagonist is any one or more of  $\text{PGF}_{2\alpha}$  dimethyl amide;  $\text{PGF}_{2\alpha}$  dimethyl amine; AL-8810 ((5Z,13E)-(9S,11S,15R)-9, 15-dihydroxy-11-fluoro-15-(2-indanyl)-16,17,18,19,20-pentanoic acid); AL-3138 (11-deoxy-16-fluoro  $\text{PGF}_{2\alpha}$ ); phloretin; glibenclamide; ridogrel; PHG113; PCP-1 (RVKFKSQHRQGRSHHEM); PCP-2 (RKAVLKNLYKLASQCCGVHVLSLHWELSSIKNSLKVAAISESPV AEKSAST); PCP-3 (CLSEEAKEARRINDEIERQLRRDKRDARRE-NH<sub>2</sub>); PCP-4 (KDTILQLNLKBYNLV-NH<sub>2</sub>); PCP-8 (ilghrdyk); PCP-10 (wedrfyll); PCP-13 (ILGHRDYK); PCP-14 (YQDRFYLL); (ILAHRDYK); PCP-13.7 (ILAHRDYK); PCP-13.8 (ILAHRDYK); PCP-13.11 (ILGFRDYK); PCP-13.13 (ILGHKDYK); PCP-13.14 (ILGHRNYK); PCP-13.18 (ILGHQDYK); PCP-13.20 (ILGHRDY-

amide); PCP-13.21 (ILGHRDYK-amide); PCP-13.22 (ILGWRDYK); PCP-13.24 (ILGXRDYK); and PCP-15 (SNVLCISIF).

6. A method according to any one of Claims 1 to 5 wherein the agent is an antagonist of  $\text{PGF}_{2\alpha}$ .
7. A method according to Claim 6 wherein the  $\text{PGF}_{2\alpha}$  antagonist is an anti- $\text{PGF}_{2\alpha}$  antibody.
8. Use of at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor, in the manufacture of a medicament for treating or preventing menorrhagia in a female individual.
9. A pharmaceutical composition comprising at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor for treating or preventing menorrhagia in a female individual.
10. A vaginal ring or a tampon or an intrauterine device comprising at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor.
11. A use according to Claim 8, or a pharmaceutical composition according to Claim 9, or a vaginal ring or a tampon or an intrauterine device according to Claim 10, wherein the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor prevents or reduces the binding of  $\text{PGF}_{2\alpha}$  to the FP receptor.
12. A use according to Claim 8, or a pharmaceutical composition according to Claim 9, or a vaginal ring or a tampon or an intrauterine device according to Claim 10, wherein the agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor affects the interaction between  $\text{PGF}_{2\alpha}$  and the FP receptor, or the interaction between the FP receptor and the associated  $G_{\alpha q}$  protein, thus inhibiting or disrupting a  $\text{PGF}_{2\alpha}$ -FP mediated signal transduction pathway.

13. A use according to Claim 8, or a pharmaceutical composition according to Claim 9, or a vaginal ring or a tampon or an intrauterine device according to Claim 10, wherein the agent comprises an antagonist of the FP receptor.
14. A use, or a pharmaceutical composition, or a vaginal ring or a tampon or an intrauterine device according to Claim 13, wherein the FP receptor antagonist comprises any one or more of PGF<sub>2α</sub> dimethyl amide; PGF<sub>2α</sub> dimethyl amine; AL-8810 ((5Z,13E)-(9S,11S,15R)-9, 15-dihydroxy-11-fluoro-15-(2-indanyl)-16,17,18,19,20-pentanor-5,13-prostadienoic acid); AL-3138 (11-deoxy-16-fluoro PGF<sub>2α</sub>); phloretin; glibenclamide; ridogrel; PHG113; PCP-1 (RVKFKSQHRQGRSHHLEM); PCP-2 (RKAVLKNLYKLASQCCGVHVISLHIWELSSIKNSLKVAAISESPV AEKSAST); PCP-3 (CLSEEAKEARRINDEIERQLRRDKRDARRE-NH<sub>2</sub>); PCP-4 (KDTILQLNLKEYNLV-NH<sub>2</sub>); PCP-8 (ilghrdyk); PCP-10 (wedrfyll); PCP-13 (ILGHRDYK); PCP-14 (YQDRFYLL); (ILAHRDYK); PCP-13.7 (ILAHRDYK); PCP-13.8 (ILaHRDYK); PCP-13.11 (ILGFRDYK); PCP-13.13 (ILGHKDYK); PCP-13.14 (ILGHRNYK); PCP-13.18 (ILGHQDYK); PCP-13.20 (ILGHRDY-amide); PCP-13.21 (ILGHRDYK-amide); PCP-13.22 (ILGWRDYK); PCP-13.24 (ILGXRDYK); and PCP-15 (SNVLCISIF).
15. A use according to Claim 8, or a pharmaceutical composition according to Claim 9, or a vaginal ring or a tampon or an intrauterine device according to Claim 10, wherein the agent comprises an antagonist of PGF<sub>2α</sub>.
16. A use, or a pharmaceutical composition, or a vaginal ring or a tampon or an intrauterine device according to Claim 15, wherein the PGF<sub>2α</sub> antagonist comprises anti-PGF<sub>2α</sub> antibodies.

ABSTRACT

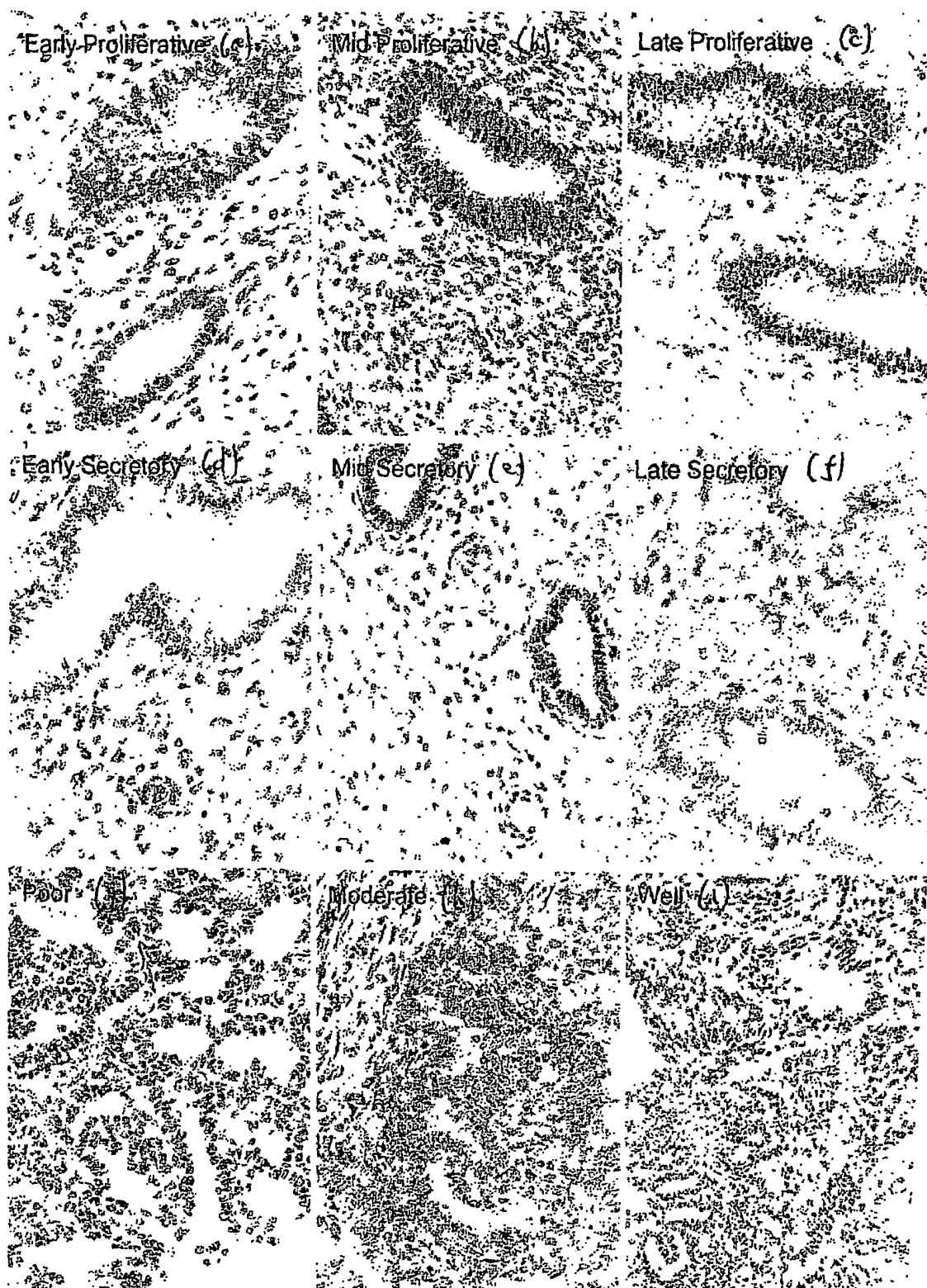
~~METHODS OF TREATMENT~~ OF MENORRHAGIA

menorrhagia

A method of treating or preventing ~~menorrhagia~~ in an female individual, the method comprising administering to the individual at least one agent that prevents  $\text{PGF}_{2\alpha}$  having its effect on the FP receptor.

~~Figure 2~~

Figure 1: FP in-situ hybridisation in human endometrium and adenocarcinoma



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Figure 2: FP mRNA expression

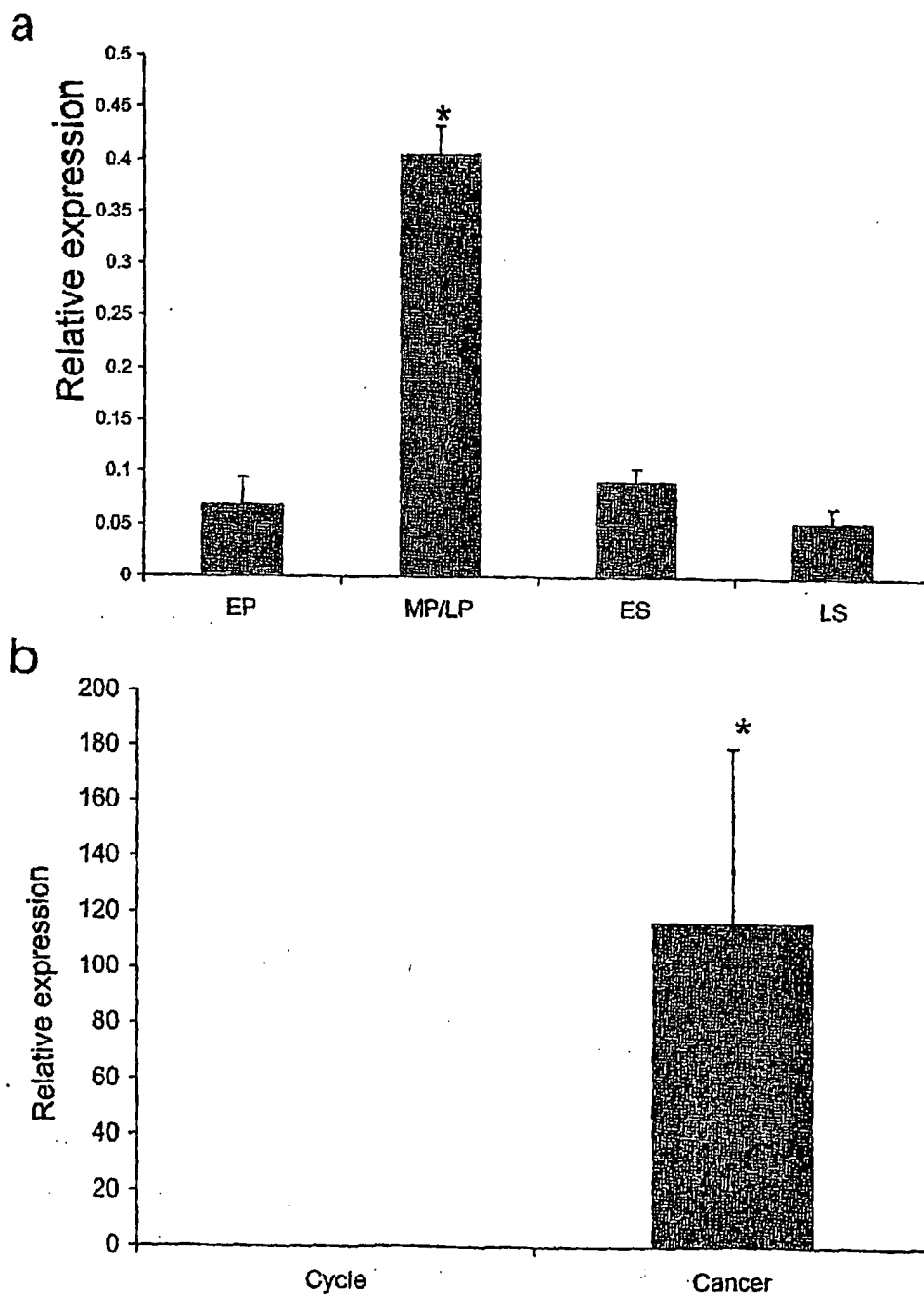


Figure 3: Total InsP mobilisation in endometrium (n=2)

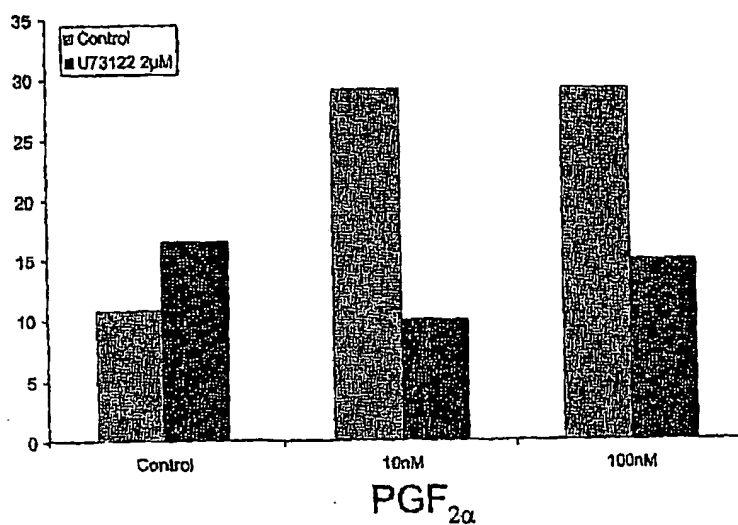


Figure 4: PGF<sub>2α</sub>-induced ERK phosphorylation

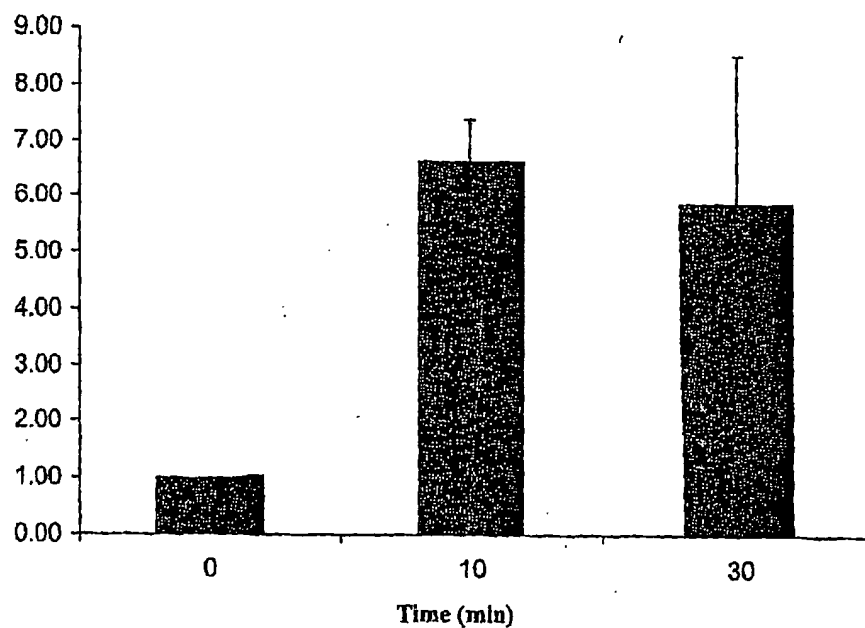
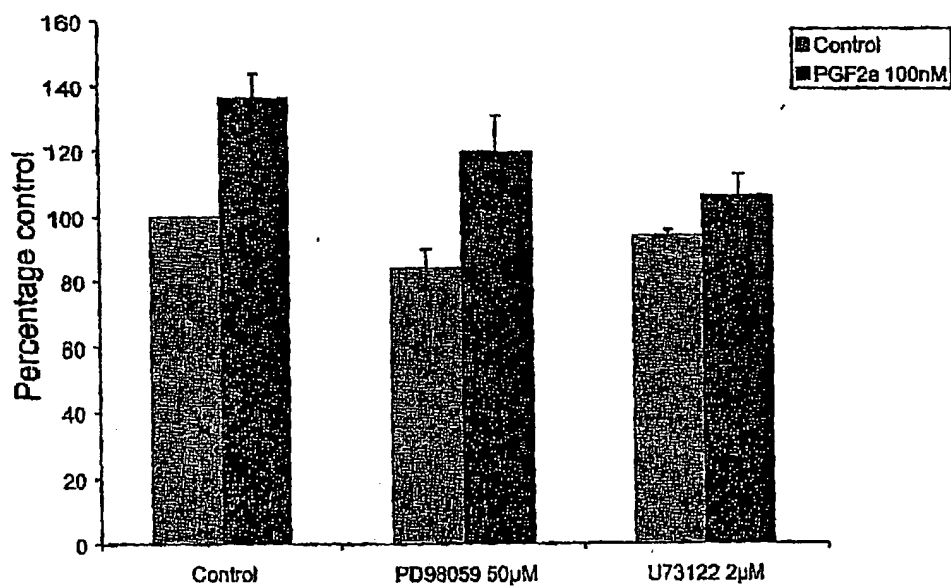




Figure 5: PGF<sub>2α</sub>-induced BrdU incorporation



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